CASE REPORTS

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Recurrent Pneumonitis Due to Pseudomonas Cepacia

—An Unexpected Phagocyte Dysfunction

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A PATIENT with necrotizing pneumonitis due to Pseudomonas cepacia (EO-I) was described previously.[1] Two years later at age 21 years, this patient again had severe pneumonia, and Ps. cepacia was isolated from bronchial washings.

The patient's phagocytes ingested but failed to kill Ps. cepacia and several other organisms. The phagocyte dysfunction was indistinguishable from that found in chronic granulomatous disease of childhood (CGD). Most patients with CGD have frequent infections, have multiple granulomatous abscesses beginning early in life, and have a short life span. In the present case, in sharp contrast to this pattern, the clinical course of the patient has been benign.

Materials and Methods

Phagocyte bactericidal capacity was determined by a modification of the method of Quie et al.[2] One milliliter of reaction mixture contained phagocytes isolated from heparinized venous blood and bacteria, in quantities noted in Table 1, suspended in Hanks' balanced salt solution containing 0.1 ml type AB Rh+ serum. After incubation at 37°C (98.6°F) for two hours in a rotating tumbler, 0.1 ml of reaction mixture was diluted with 0.9 ml of distilled water to lyse leukocytes; after culture in pour plates for 12 hours, colonies were counted. Viable organisms remaining in a mixture containing serum, known concentrations of bacteria, and leukocytes before and after two hours' incubation were counted after lysis of cells with sterile water.

Phagocyte nitroblue tetrazolium (NBT) reduction was quantitated by the method of Baehner and Nathan[3] except that instead of fibrinogen for red cell sedimentation, one volume of 6 percent dextran in sterile 1 percent phosphate-buffered saline solution, pH 7.4, was added to ten volumes of blood.

Leukocyte fractionation and enzyme assays were assessed by the methods of Holmes et al.[4] Formate oxidation (H_2O_2 production) was measured by the technique of Holmes, Page and Good.[5]

Some enzyme assays and determinations of NBT reduction were done on blood specimens that had been drawn six to ten hours earlier and transported at airplane cabin temperatures. Activities of control specimens handled in the same fashion were not different from those of fresh normal blood. Normal control specimens were taken from adult laboratory and hospital personnel.

Case Summary

Before age 5 years, the patient had frequent respiratory infections with high fever. On four occasions, brief periods in hospital and penicillin therapy resulted in prompt resolution of fever.

He was subsequently healthy until age 19 years, when pneumonitis due to Ps. cepacia developed. The lungs cleared after several weeks' therapy with chloramphenicol.[1] He was then well until 24 months later when symptoms of upper respiratory infection developed in him and in others in his family. Family members recovered promptly; but the patient had progressively more severe chills,

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fever, cough and pleuritic chest pain. An x-ray film of the chest showed a left lower lobe infiltrate. Treatment for one week with penicillin and cephloridin brought no improvement and he was transferred to the University of Oregon Medical School Hospital.

No other family member has had serious or recurrent infections.

The patient was acutely ill, with a painful nonproductive cough. He appeared well developed and well nourished, and there was no evidence of chronic debilitation. Blood pressure was 112/60 mm of mercury, pulse 120 per minute and regular, respiratory rate 20 per minute, and temperature 40.6°C (105°F). Mucous membranes of the pharynx were dry but not erythematous. Lymph nodes were normal to palpation. The left hemidiaphragm was elevated. Decreased breath sounds and occasional crepitant and fine inspiratory rales were heard over the left lower lung. No friction rub was heard. Results of examination of the heart and peripheral vessels were within normal limits. The liver, spleen and kidneys were not palpable. No skin lesions were noted. Neurological examination results were normal except for slight obtundation and irritability.

The hematocrit was 40 percent and leukocytes numbered 4,150 per cu mm with 63 percent polymorphonuclear neutrophils, 14 percent band forms, 13 percent lymphocytes and 10 percent monocytes. Thrombocytes were present in normal numbers. The urine was slightly cloudy with a pH of 5, specific gravity of 1.023 and 3+ albumin. Microscopic examination showed 5 to 10 pus cells per high-power field and an occasional red cell, but no casts. The blood urea nitrogen was 17 mg per 100 ml and the creatinine clearance was 95 ml per minute. Quantitative urine protein was 100 mg in 24 hours. The serum glutamic oxaloacetic transaminase (SGOT) was 162 units (normal, 15 to 45), bilirubin 0.7 mg per 100 ml, prothrombin-proconvertin 100 percent of normal, and bromsulfalein retention 4 percent in 45 minutes.

Skin tests for tuberculosis, histoplasmosis, coccidioidomycosis and blastomycosis were negative, but normal cellular immunity was suggested by a positive mumps skin test. Cold agglutinins were positive in a titer of 1:32. The patient's ability to form humoral antibodies was shown by the presence of low titers of salmonella Groups C and E, brucella, and Weil-Felix antibodies. Serum IgG was 2,000 (normal, 800 to 1,800), IgA 560 (normal 90 to 450), IgM 90 (normal 60 to 250), and group β_1C complement 570 mg per 100 ml (normal 80 to 140).

An x-ray film of the chest on admission showed an infiltrate in the postero-lateral segments of the left lower lobe. The films were strikingly similar to those taken two years before (Figure 1).

Sputum, nasopharyngeal and blood cultures grew no pathogenic organisms. At bronchoscopy, segmental bronchi of the left lower lobe were observed to be inflamed and edematous, and pus obtained from this area grew Ps. cepacia. Both in its cultural and biochemical characteristics it was similar to the organism isolated from the patient's sputum in 1967. Results of *in vitro* antimicrobial disc susceptibility tests were identical in both isolates. They were sensitive to chloramphenicol and kanamycin, intermediately sensitive to tetracycline and resistant to ampicillin, cephalothin, streptomycin, polymyxin and penicillin. The Ps. cepacia isolated in 1969 was also found to be resistant to gentamicin.

In view of the patient's critical condition and previous response, he was given cloramphenicol, 12 grams a day. Within 36 hours, the temperature had returned to normal. Over the ensuing ten days, chest findings and x-ray showed pronounced improvement. The SGOT and urine abnormalities disappeared. Chloramphenicol was decreased to 6 grams a day on the third day, and to 2 grams a day on the seventh day. This dose was maintained for 24 days, although serum inhibitory activity (SIA) determinations showed no inhibition of growth in undiluted serum. On the basis of disc sensitivity tests, kanamycin was given for ten days (day 22 to 32) but was discontinued because adequate SIA could not be obtained.

We considered use of carbenicillin, a drug known to be active against Pseudomonas organisms. The minimum inhibitory concentration (MIC) determined by the broth dilution method was found to be 6.2 μg per ml and disc diffusion tests showed a zone of 28 mm around a 100 μGm carbenicillin disc. Since adequately high serum levels can easily be achieved with carbenicillin,[6] the patient received 6 grams a day intravenously from day 32 through discharge on day 39.

The patient left the hospital without symptoms and without physical findings of residual lung disease. An x-ray film of the chest showed no abnormality except for scarring and elevation of the left diaphragm as shown in Figure 1.

In follow-up by the patient's family physician to the present time no new findings or subsequent

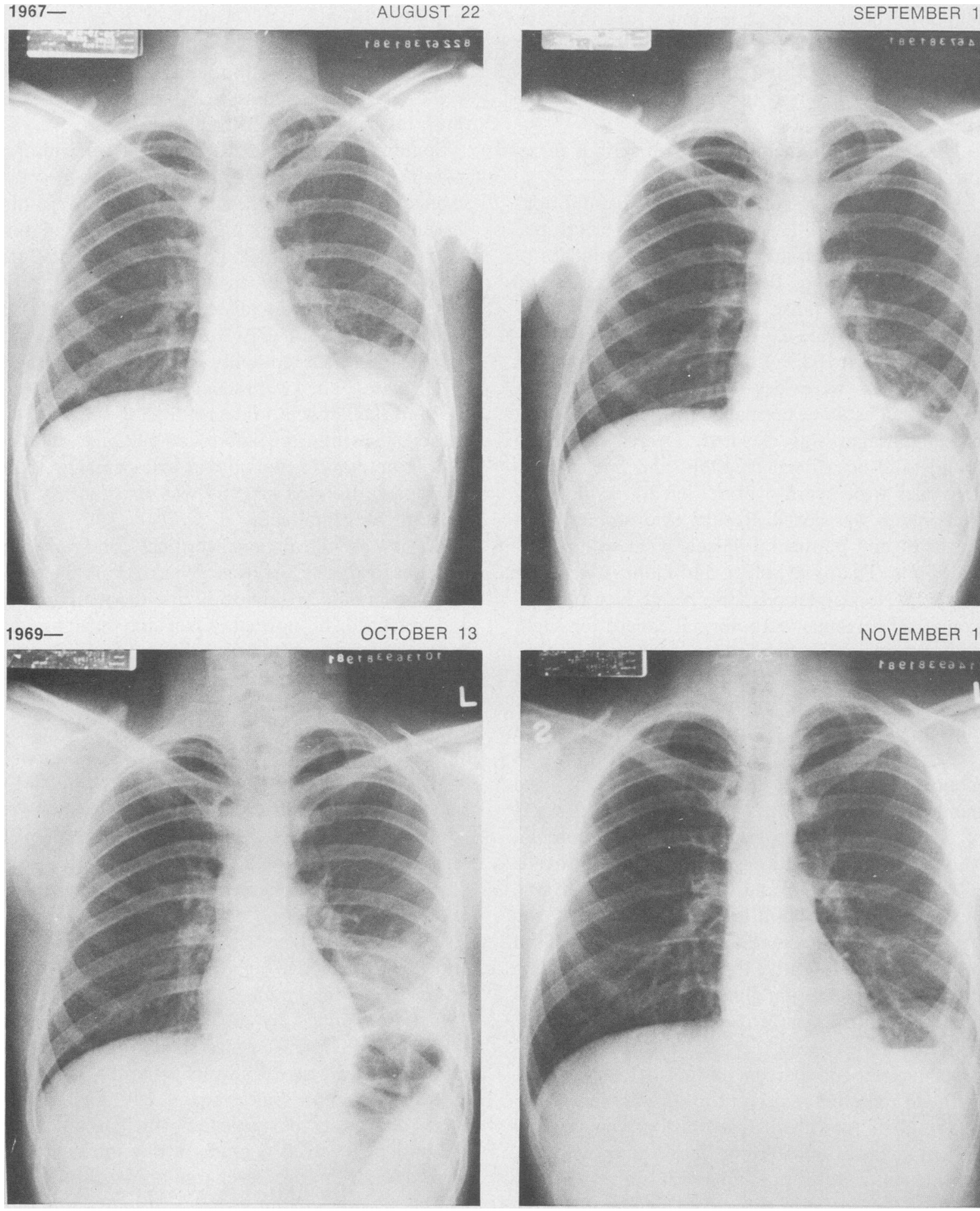

Figure 1.—Admission and discharge chest x-ray films showing similar left lower lobe infiltrates which cleared in each case after chloramphenicol therapy.

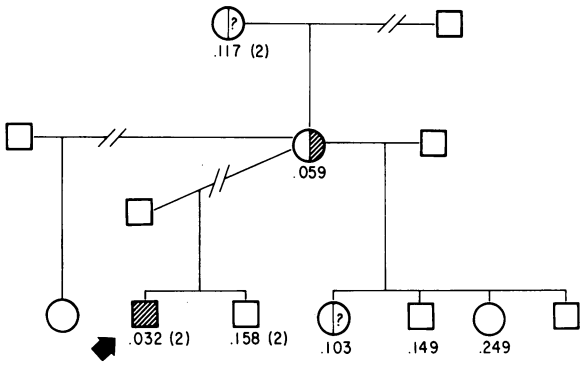

Chart 1.—Family pedigree and nitroblue tetrazolium reduction tests. Patient indicated by arrow. Figures represent net change in optical density $\lambda_{515}/2.5\times10^6$ phagocytes, with number of determinations in parenthesis. Normal controls (N=34) averaged 0.206, range 0.153-0.425. —/ /—=divorce.

serious illness of any kind has been observed. The patient appears to have fully recovered.

Additional Studies

Quantitative NBT reduction (Chart 1) by the patient's leukocytes was decidedly low on hospital day 38, and was unchanged three months later. Values in his mother were intermediate, and those of his maternal grandmother and of a half-sister were near but below the lowest normal values observed in our laboratory.

Bactericidal studies (Table 1) showed that the patient's leukocytes failed to kill Ps. cepacia, Ps. aeruginosa, E. coli and S. aureus.

Phagocytosing H_2O_2 production, measured as formate oxidation, was subnormal (Table 2). Patient phagocytes, incubated with latex alone for 15 minutes, engulfed 5 to 20 particles per cell, showing that phagocytosis was normal. Activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the patient's leukocytes were normal.

Review of biopsy material from the lung obtained during the patient's first admission showed necrotizing granulomas (Figure 1).[1] No pigmented histiocytes were found.

After the patient was discharged from the hospital, a second strain of Ps. cepacia highly resistant to carbenicillin was identified in the original culture.

Discussion

It was difficult to decide on the right therapy for this patient. His condition did respond to massive doses of chloramphenicol without serious side

TABLE 1.—*Comparison of Leukocyte Bacterial Capacity in Patient and a Normal Control*

Organism	Zero Time Control	120 Minutes Subject Cells	120 Minutes Control Cells
S. aureus*	6.3	6.2 (98)	3.5 (55)
Ps. aeruginosa*	3.35	3.3 (98)	1.1 (33)
E. coli*	6.05	5.8 (96)	2.35 (39)
Ps. cepacia (EO-I)†	1.4	0.9 (64)	0.05 (3)

Results expressed as numbers of viable bacteria $\times 10^6$/ml of incubation mixture before (zero time) and after (120 min) incubation. The zero time mixture contained no phagocytes. Figures in parentheses represent percent of zero time control bacteria surviving at 120 minutes.
*1.0×10^6 phagocytes in 1 ml incubation mixture.
†2.0×10^6 phagocytes in 1 ml incubation mixture.

TABLE 2.—*Phagocyte 14C-Formate Oxidation*

	Subject Cells	Control Cells
Number of Determinations	2	12
Resting	0.26*	0.46±0.16†
Phagocytosing	0.36‡	1.19±0.28

*Nanomoles $CO_2/10^7$ phagocytes/40 min.
†Mean ± S.D.
‡$p<.005$

effects. However, the severity of illness and the high likelihood that the patient was still infected and susceptible to reactivation of life-threatening disease led some consultants to recommend lobectomy on the infected lung. We elected not to do this because of the likelihood that there was both upper and lower lobe involvement that would require pneumonectomy which could be carried out at a time when he was clinically well. Furthermore, we were hesitant to cross pleural barriers in a patient who had clinically recovered from acute infection.

Therapeutic concentration of kanamycin could not be achieved, making it an unsatisfactory backup drug should he once again become ill. Carbenicillin appeared promising on the basis of disc sensitivity studies, but SIA was not determined. Even with an adequate serum inhibitory level, the drug may not penetrate into diseased lung and granulomas. Unfortunately, Pseudomonas strains may rapidly become resistant to carbenicillin therapy.[7] Furthermore, we were able to separate a resistant mutant in his original culture, suggesting that, even with adequate tissue levels, survival of organisms remained a possibility. Two recent reports[8,9] demonstrated that combinations including trimethoprim (TMP) and sulfamethoxazole are active against Ps. cepacia. TMP-sulfamethoxazole was used for treatment of Ps. cepacia endocarditis and in one case[9] it was successful in sterilizing the patient's blood and aortic valve.

In the present case, the unusual infections seem

clearly related to the patient's leukocyte dysfunction. His mild clinical course sets him apart from other patients with CGD. CGD is characterized clinically by severe recurrent infections with bacteria which are often of low virulence, widespread suppurative granulomas containing lipid-laden macrophages, and sex-linked inheritance. Clinical variations among patients with CGD have been noted.[10-14] Only one patient, Case 8 of Thompson and Soothill, approaches our patient in paucity of infections.[11] That patient, an 11-year-old boy, had chronic skin sepsis during his first year, a groin abscess at age three years and pneumonia twice at age ten years. Of five patients living to ages 13 to 20 years, all had abdominal visceral abscesses in addition to many other infections.[11-14]

CGD phagocytes have subnormal post-phagocytic increments in H_2O_2 production[5] and NBT reduction.[3] They do not efficiently kill staphylococci and certain Gram-negative bacilli, organisms which produce no net H_2O_2.[15] In these respects, our patient's phagocytes are quantitatively as abnormal as other CGD phagocytes. However, these determinations do not directly measure the activity of the undefined abnormal gene product(s) responsible for CGD. Leukocyte glutathione peroxidate deficiency[4,16] (which is clinically different from CGD only in its autosomal recessive inheritance) and profound G-6PD deficiency are two of possibly several enzyme deficiencies which make up the CGD syndrome.

Variation in clinical and laboratory manifestations of genetically determined disorders is determined by differences in kinds of mutation and in modifying factors, including environment. The causes of clinical variation among patients with CGD and glutathione peroxidase deficiency are not yet established, but extreme variation obviously occurs. Our patient's relatively infrequent severe infections may be due to a less than disastrous mutation. This case suggests that leukocyte dysfunction should be considered in any patient, regardless of age, who has unusual infections.

Summary

Recurrent pneumonitis due to Pseudomonas cepacia (EO-I) developed in a previously healthy patient. Phagocyte bactericidal, peroxide-generating and nitroblue tetrazolium-reducing capacities were decreased. A relatively benign history and the good health of the patient at age 22 years set this case apart from other cases of chronic granulomatous disease.

Addendum

Shortly after this manuscript was submitted, the patient was readmitted to the University of Oregon Health Sciences Center with a recurrence of severe left lower lobe pneumonitis. The history, physical and radiological findings were remarkably similar to those in the previous two episodes. Mild phagocyte dysfunction was again demonstrated. No pathogen could be isolated from sputum, bronchial washings, blood cultures or material from needle aspiration of the lung. In the face of a rapidly deteriorating course, his physicians instituted chemotherapy consisting of cephalothin, gentamycin and carbencillin. Within 36 hours the patient's temperature returned to normal and the pneumonitis resolved rapidly over the ensuing five days. The patient has returned to Alaska and is currently asymptomatic.

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